United States Environmental Protection Agency

LABORATORY ANIMAL PROJECT REVIEW

Please note:

1. All information in this LAPR is considered privileged and confidential by the IACUC and regulatory authorities.
2. Approved LAPRs are subject to release to the public under the Freedom of Information Act (FOIA). Do not include proprietary or classified information in the LAPR.
3. An approved LAPR is valid for three years.

LAPR Information

LAPR Title:	Pharmacokinetic evaluation of Perfluoroalkyl acid (PFAA) in the mouse
LAPR Number:	17-08-003
Principal Investigator	Exemption 6
Author of this Document:	Exemption 6 RTP/USEPA/US
Date Originated:	08/12/2014
LAPR Expiration Date:	08/31/2017
Agenda Date:	08/27/2014
Date Approved:	09/16/2014
Date Closed:	07/31/2017

APPROVALS

APPROVER	NAME	APPROVAL DATE	COMMENTS	
	Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	09/16/2014	DMR	
	Exemption 6 Exemption 6 Exemption 6 /RTP/USEPA/US by Exemption 6 /RTP/USEPA/US	09/16/2014	DMR	

Administrative Information

1. Project Title (no abbreviations, include species):
Pharmacokinetic evaluation of Perfluoroalkyl acid (PFAA) in the mouse

Is this a continuing study with a previously approved LAPR?
No

2. What is the Intramural Research Protocol (IRP) number covering this project?
IRP-NHEERL-RTP/RTD/EB/Exemption 6/05-01-000; CSS 4.2.2, Sciences approaches, tools and data for informing cumulative risk assessment and risk management of high priority classes/groups of chemicals.

3. EPA Principal Investigator/Responsible Employee:

Principal Investigator	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6/Exemption 6/Exemption 6 RTP/USEPA/US	TAD *Branch* DTB	MD B105-04

4. Alternate Contact:

Alternate Contact	*Phone Number*	*Division*	*Mail Drop*
Exemption 6	Exemption 6 *Lotus Notes Address* Exemption 6/Exemption 6/Exemption 6/Exemption 6 RTP/USEPA/US	TAD *Branch* DTB	MD B105-04

SECTION A - Description of Project

1. Study objectives, presented in non-technical language such that it is understandable by non-scientific persons, including how the study addresses health protection. If this is a continuing study from a previous LAPR, briefly justify the continuation. Please spell out all acronyms and abbreviations with their initial use.

The objective of the proposed study is to complete the pharmacokinetic profiling of a class of environmental contaminants called perfluoroalkyl acids (PFAAs) that are composed of sulfonic acids, carboxylic acids and phosphonic acids, each with various carbon-chain lengths ranging from 4-14 (C4-C14). The research goal is to fill data gaps and reduce the uncertainties associated with risk assessment of these chemicals. PFAAs are used extensively in industry and consumer products (such as water- and stain-resistant textiles and grease-proof paper products for food packaging), found to be ubiquitous and persistent in the environment, and detectable in the general population. Risk assessments of these chemicals are presently conducted by Office of Water (OW), Office of Pesticides, Prevention and Toxics, (OPPT)) as well as other state regulatory agencies (e.g. New Jersey,

Minnesota, North Carolina) to set values for health advisories. Because these PFAAs are found in the environment and most of them are detectable in humans and wildlife, the Agency must consider the health risks of these chemicals in toto.

Toxicities of PFAAs are in large part driven by their pharmacokinetic (PK) properties, i.e. how fast the chemicals are eliminated. There are major species differences in the rate of chemical clearance among rat, mouse, monkey and humans, and between sexes. A good deal of PK data have been generated by our labs and others for a number of environmentally relevant PFAAs (perfluorobutanoic acid (PFBA), perfluorohexanoic acid (PFHxA), perfluorooctanoic acid (PFOA), perfluorononanoic acid (PFNA), perfluorobutane sulfonate (PFBS) and perfluorooctane sulfonate (PFOS)) and species (rat, mouse, monkey and humans), with a notable exception of PFBS in mouse. To facilitate the extrapolation of animal data to human health risks, computational models are being constructed with existing data to make prediction on other PFAAs that lack data. One such model will be constructed by NCCT scientists to extrapolate the clearance rates among various PFAAs, among species and between sexes. To fill the data gap for this computational model, a description of PK properties of PFBS in mouse is required.

2. Scientific rationale for proposed animal use.

a. Why is the use of animals necessary?

Use of animals is absolutely necessary in this project because, to date, no suitable alternative method or model is available to evaluate the pharmacokinetic disposition of chemicals. We shall use existing data of related PFAAs to guide our dose selections and treatment regimens.

b. Justify the species requested:

The mouse provides an appropriate model for human health risk assessment of PFAAs and related chemicals because the PK profiles in mice (for those PFAAs evaluated thus far) resemble those seen in humans, unlike the major sex difference noted in rats (but not humans). For this project, mouse data for PFBS are missing for the construction of a computational PK model

3. How was it determined that this study is not unnecessary duplication?

We have coordinated closely with the Program Offices as well as the industry to assure that our efforts are unique and not duplicative. Literature searches with Pubmed and Toxline using terms such as "pharmacokinetic and perfluorinated chemicals" show that this proposed research has not been performed by any other laboratory.

SECTION B - In Vivo Procedures

1. Briefly describe experimental design. Supplementary information may be attached at the end of the LAPR, but please include critical information within the body of the LAPR.

Adult male and female mice (6-8 weeks of age) will be given PFBS once by oral gavage for rate of elimiation determination. Animals will be killed by decapitation at intervals of 1h, 2h, 4h, 8h, 16h, and 24 h after treatment. For the 24h time point, mice will be housed singly in metabolic cages for urine and feces collection. 3 males and 3 females treated with water vehcile will be kept in the animal room for 24h before euthanasia to serve as background control (PFAAs are known to be ubiquitous). Serum, urine, feces, liver and kidney will be collected for chemical analysis. PFBS is not known for its acute toxicity, but overt signs of toxicity such as hunching, scruffy coat, dehydration, lethargy will be monitored for 24 hours

2. Justify the number of animals. Include explanation (e.g., biological, statistical, regulatory rationale) for the number of animals needed for each treatment group, and the overall number requested for the duration of the LAPR.

Our study design requires two dose levels to determine if pharmacokinetics of PFBS is linear (with exposure doses). PFBS is expected to have a fast clearance rate; thus multiple (6) time intervals of evaluation within 24h are needed. Based on our previous experience, a minimum of 3 animal per sex per dose per time point is required for a reliable PK determination.

(3m+3f) x 6 time points x 2 doses + 6 controls (3m + 3f) = 78

Should statistical analysis reveal large variance among mouse samples, an amendment will be submitted to request additional animals for investigation.

3. State how many animals over the study period are expected to be used under the following three categories of pain/distress (USDA nomenclature as defined in the instructions): Please enter numbers only.

Categories	Adults	Offspring
C) Minimal, transient, or no pain/distress:	78	0
D) Potential pain/distress relieved by appropriate measures:	0	0
E) Unrelieved pain/distress:	0	0

4. For tracking purposes, please check if this LAPR includes any of the following:

☐ Restraint (>15 Minutes) ☐ Survival surgery
☐ Food and/or water restriction (>6 Hours) ☐ Non-survival surgery

5. Category C procedures. Describe each procedure separately, include details on the following:

a. Treatments (e.g., dosages, duration of exposure, route, volume, frequency):
Two doses (30 and 300 mg/kg) will be evaluated for linear pharmacokinetics. Both male and female adult mice will be treated once by oral gavage to determine sex difference. PFBS will be prepared in distilled water and each mouse will receive a volume of 10 ml/kg. Background control will receive distilled water. Disposable and pliable plastic needles, 20Gx30mm, will be used for the oral gavage.

b. Survival Blood Collections (method, volume, frequency):

c. Testing methods (including non-stressful dietary restrictions/modifications, mild non-damaging electric shock):

d. Animal restraint and confinement beyond routine housing and handling. Include a description of the type of restraint device, acclimation to device, duration of restraint:

e. Breeding for experimental purposes (e.g. length of pairing, number of generations):

f. Describe how animals will be monitored (e.g., frequency of observations, by whom):
Animals will be monitored periodically by lab staff (Exemption 6) for 24 hours after a single oral gavage

6. Non-surgical Category D or E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9).

a. Treatments (e.g. dosages, duration of exposure, route, volume, frequency):

b. Survival Blood Collection (method, volume, frequency):

c. Testing methods:

d. Restrictions placed on the animals' basic needs (e.g., food and/or water deprivation, light cycles). Provide details regarding the length of deprivation:

e. Describe how animals will be monitored (e.g., frequency of observations, by whom):

f. Analgesia (Category D Procedures) - list drugs, dosages, route of administration and frequency:

g. If treatment-related deaths are expected, this must be thoroughly justified. Death as an endpoint is highly discouraged:

7. Surgical Category D and E procedures. Describe each procedure separately, include details on the following (Also fill in Section B.9)

a. Complete description of surgical procedure including presurgical preparation, aseptic technique, surgical closure, etc:

b. Anesthetic regimen (drugs, dosages, volume, and route of administration). The use of paralytic or neuromuscular blocking agents without anesthesia is prohibited:

c. Postoperative care (thermal support, special feeding, frequency and duration of monitoring, responsible personnel, removal of sutures/staples):

d. Post operative analgesics (drugs, dosage, and volume and route of administration, frequency):

e. Will any animals be subject to more than one major surgical survival procedures?
◯ Yes ◯ No
f. Identify any surgical procedures performed at other institutions or by vendors:

8. Humane interventions (for treatments/procedures in all categories).

a. Describe actions to be taken in the event of expected or unexpected deleterious effects from procedures or chemical exposures.
Doses of chemicals chosen for evaluation in this study are low, and no overt toxicity is expected. If signs of overt toxicity (such as hunching, scruffy coat, dehydration or lethargy) are found, staff veterinarian will be consulted for treatment and/or course of action taken, possibly including euthansia.

b. State criteria for determining temporary or permanent removal of animals from the study.
For all animals, evaluation of coat and skin conditions, grooming behaviors, weight changes, dehydration and/or discomfort will be performed. If these signs of ill health are noted, the attending veterinarian will be consulted for further course of action that may include euthanasia.

9. Alternatives to pain and distress (Category D and E Procedures only). Provide narrative regarding the sources consulted to ascertain whether acceptable alternatives exist for potentially painful/distressful procedures. Include databases searched or other sources consulted, the date of the search and years covered by the search, and key words and/or search strategy used. Assistance with searches is available through the EPA Library Staff.

SECTION C - Animal requirements

Describe the following animal requirements :

1. Indicate the number of animals required over the study period for this protocol. Please enter numbers only.

a. Animals to be purchased from a Vendor for this study:	78
b. Animals to be transferred from another LAPR:	0
LAPR Number that is the source of this transfer:	
c. Animals to be transferred from another source:	0
d. Offspring produced onsite (used for data collection and/or weaned):	0
e. TOTAL NUMBER of animals for duration of the LAPR	78

2. Species (limited to one per LAPR): Mouse/Mice

3. Strain: CD-1 mouse/mice

Describe special requirements for animals with altered physiological responses

(e.g., genetically altered, aged)

4. Sources of animals:
Charles River as vendor for CD-1 mice.

5. Provide room numbers where various procedures will be performed on animals:
All procedure will be in room Exemption 6

6. Will any animals be housed in areas other than the animal facility longer than 12 hours? If so, state location. Such areas require prior IACUC approval as a satellite facility before LAPR can be reviewed.
No *Room Numbers:*

7. Describe any transportation and containment methods involved in moving animals between EPA buildings, or between EPA and other institutions (excluding any commercial shipments)
None

8. Describe any unusual housing or husbandry requirements, or acclimation requirements. Justify any treatment beginning less than 3 days after arrival.
For the 24h time point, mice will be placed in metabolism cages individually for urine and feces collection after treatment, no acclimation to metabolism cage housing is required.

9. Describe special assistance requested of the animal contract staff, including procedures and dosing. NOTE, this request must be submitted separately to the Animal Resources Program Office (ARPO)

10. Housing and Enrichment.
The IACUC encourages the use of environmental enrichment whenever possible (see IACUC website for details). Provide details on how the animals will be housed, including type of cage (e.g., solid bottom or wire screen), bedding material, number of animals per cage, and environmental enrichment. Note that housing rodents individually without environmental enrichment requires justification.
Upon arrival, mice will be group-housed (3/cage) in solid bottom cages with pine shavings and nestlets for environmental enrichment. All mice will be acclimated for 5 or more days until the day of experiment. Except for mice assigned to the 24h time point, mice will remain group housed. For the 24h time point, mice will be singly housed in standard metabolism cages after dosing for individual urine and feces collection. No acclimation to metabolism cage housing is required.

SECTION D - Agents Administered to Animals

1. Identify all hazardous and non-hazardous agents to be administered to living animals. For agents requiring a Health and Safety Research Protocol (HSRP), provide the title of the approved HSRP for each such agent. If no protocol is required for an agent deemed potentially hazardous (e.g. nanoparticles, recombinant DNA), describe the safety precautions to be used.
Provide maximum dosing levels and route-appropriate LD50s (where available) for each agent used for dosing.
Oral LD50 for PFBS in rat: >2000 mg/kg, maximum dose: 300 mg/kg/day (once)

Standard safety procedures involving disposable gloves, labcoat, and safety glasses will be routinely practiced when these chemicals are handled.

2. Describe any plans to administer human or animal tissues, blood or body fluids to the animals in this LAPR, and provide:

a. Information to assure that such material is pathogen-free

b. A statement regarding any safety precautions necessary for handling the material.

NOTE: Any unresolved health/safety questions which arise during IACUC review of this LAPR will require consultation with the Safety, Health, and Environmental Management Office.

SECTION E - Personnel Training and Experience

1. Identify all project personnel conducting animal experimentation. Specify the techniques for which they have responsibility, and their relevant training and experience. Additional personnel may be added to the table below as a group (by Division) for Category C procedures. By so doing you are giving assurance that these personnel have received all required training and are qualified to perform the Category C techniques requested.

Use this area to type in additional personnel information not available in the table drop-down lists:

Hint: *The names in the first 2 lines of the table below are filled automatically from the Principal Investigator & Alternate Contact fields. A new line will be made available when a name is selected & upon leaving the name field (i.e. tabbing or clicking in another field).*

NAME	ROLE	SPECIFIC RESPONSIBILITY	RELEVANT TRAINING
Exemption 6	Principal Investigator	Study design, animal treatment and necropsy	EPA NHEERL Trained, over 30 years of experience
Exemption 6	Technical Staff	Animal treatment and necropsy	EPA NHEERL Trained, over 15 years of experience
Exemption 6	Technical Staff	Animal treatment and necropsy	EPA NHEERL Trained, over 15 years of experience
RTP-NHEERL	Tech Support	Category C Procedures	EPA IACUC Trained

SECTION F - Animal Breeding Colonies

This section pertains to the breeding of animals for maintenance of ongoing animal colonies. Do not include breeding that is part of experimentation and accountable under Section C.

Describe:

1. Estimated number of breeding pairs and liveborn per year

2. Breeding protocols and recordkeeping

3. Methods for monitoring genetic stability

4. Disposition of all offspring and retired breeders that are not used in accordance with the procedures described in this LAPR

SECTION G - Euthanasia

1. When will the animals be euthanized relative to experimental procedures?

12 Mice will be euthanized at 1h, 2h, 4h, 8h, 16h, and 24h after single treatment of perfluorobutane sulfonate (PFBS). Animals will be decapitated using a guillotine, with blades sharpened annually or earlier if needed. A back-up guillotine will be available.

2. Describe the euthanasia techniques:

Method(s): Decapitation
Agent(s):
Dose (mg/kg):
Volume:
Route:

Source(s) of information used to select the above agents/methods:

Common method for euthanasia

3. Provide justification and references for any euthanasia agent or method that is not consistent with recommendations of the 2007 American Veterinary Medical Association (AVMA) Guidelines for Euthanasia (e.g., cervical dislocation or decapitation without anesthesia; cervical dislocation in rodents weighing more than 200 grams).

In compliance with 2013 AVMA Guidelines.

4. Describe how death is to be confirmed.

Vital organ section

SECTION H - Disposition of Used and Unused Animals

Describe the disposition of any animals remaining after project completion.

None

The IACUC encourages investigators to reduce the overall number of animals used at NHEERL. Would you consider transferring any unused animals from this LAPR to another approved LAPR?

● Yes ○ No

SECTION I - Assurances

1. Animals will not be used in any manner beyond that described in this application without first obtaining formal approval of the IACUC.
2. All individuals involved in this project have access to this application, are aware of all EPA policies on animal care and use, and are appropriately trained and qualified to perform the techniques described.
3. The proposed research using animals does not unnecessarilty duplicate any previous experimentation.
4. Thorough consideration of the three "R"'s (Replacement, Reduction, Refinement) has been given, as applicable, to a. the use of animals, and b. procedures causing pain or distress (with or without analgesia/anesthesia), including death as an endpoint. The minimum number of animals required to obtain valid experimental results will be used.
5. The Attending Veterinarian has been consulted in regard to any planned experimentation involving pain or distress to animals.
6. All procedures involving hazardous agents will be conducted in accordance with practices approved by the Safety, Health, and Environmental Management Office.
7. Individuals from outside of EPA who are collaborating on this project, and who conduct related experimentation on EPA procured or bred animals in their respective Institutions, have the equivalent of a current IACUC approved LAPR at their respective Institutions.
8. The IACUC has oversight responsibilities for animal care and use, and may request consultation

or feedback regarding the conduct of in vivo procedures, progress and accomplishments, and any problems encountered.

EPA Principal Investigator	Certification Signature Date
Exemption 6 by Exemption 6	08/19/2014

Submitted: 08/19/2014

Certification:

Certification by EPA Supervisor (Branch Chief or Division Director) that the project described herein has been reviewed and approved on the basis of scientific merit:

Branch Chief/Division Director	***Approval Date***	***Phone Number***	***Division***	***Mail Drop***
Exemption 6	08/19/2014 by Exemption 6 RTP/USEPA/US	Exemption 6 ***Lotus Notes Address*** Exemption 6 RTP/USEPA/US	TAD ***Branch*** NB	MD ***Submitted to Branch Chief for Approval*** 08/19/2014 12:48 PM

ATTACHMENTS

17-08-003 PI resp.pdf

Actions

First Update notification sent: 07/02/2015
Second Update notification sent:
First 2nd Annual notification sent:
06/29/2016
Second 2nd Annual notification sent:
07/27/2016
1st Expiration notification sent:
2nd Expiration notification sent: 07/31/2017

History Log: